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Twenty-fifth day of February 2000

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PROVISIONAL SPECIFICATION

Invention Title:

Improved microbial products

The invention is described in the following statement:

Technical Field

The present invention relates to utilisation of prebiotic and probiotic preparations and products including food, feed, nutraceutical and pharmaceutical products containing probiotic microorganisms, and products fermented by added microorganisms.

Background Art

Probiotic products, for example powders, tablets and capsules are orally administered for improving health. Similarly foods are consumed not only for sustenance but also for added health benefits such as through the addition of probiotic microorganism. Animal feeds are also being prepared with added probiotic microorganisms in order to assist the growth and performance of animals. Recent trends of consuming probiotic compositions for health benefits has led to the use of probiotic microorganisms in a variety of preparations as well as inclusion into a wide range of processed food and feed products, including processed milk-based products. Microorganisms are also added as starter cultures in order to produce a range of fermented foods e.g. milk, meat, vegetable products.

As used in this specification, a probiotic or probiotic microorganism is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. This is the definition provided by R. Fuller (AFRC Institute of Food Research, Reading Laboratory, UK) in - Journal of Applied Bacteriology, 1989. 66, pp. 365-378 "Probiotics in Man and Animals - A Review", and has subsequently been extended to include supplements and food for humans.

The constitution and quantity of the gut microflora can be influenced by conditions or stress induced by disease, life style, travel, and other factors. If microorganisms which positively effect the health and well being of the individual can be encouraged to populate the large bowel, this should improve the physiological well being of the host.

The introduction of beneficial microorganisms, or probiotics, is normally accomplished by the ingestion of the organisms in foods, drinks, fermented dairy products such as yoghurts, capsules, confectionary and other forms in such a way that the organism arrives in a viable condition in the large bowel.

One problem with the inclusion of probiotic microorganisms into processed food products is that the microorganisms often cannot survive in

the food product for any length of time. During production and storage of the food products, there is often a substantial decrease in the numbers of viable microorganisms. For example, usual shelf-life of milk-based products is calculated on the period of time before spoilage of the product. When probiotic microorganisms are added to these products, the shelf-life stated for the product may not be applicable with regard to delivering the desired number of microorganisms to the gut to obtain the required beneficial effect.

The present inventors have made the surprising discovery that the inclusion of resistant starch, in particular resistant starch in the form of, or derived from, high amylose starches containing dietary fibre, in the growth medium for the probiotic microorganisms can increase growth and yield of the microorganisms, as well as increase the survival of microorganisms in probiotic preparations or starter cultures and in food and feed products during production and over the shelf-life of these products, and improve rate of survival of the probiotic microbes during transit through the digestive tract. Dietary fibre is defined as measured by the AOAC (Association of the Official Analytical Chemistry) International Method 991.43 Official Methods of Analysis 16th Ed, The Association, Arlington VA, USA. Further addition of the resistant starch to the probiotic preparation after growth also further enhances robustness of the microbes, thus leading to enhanced survival of the probiotic microorganisms. The discovery is also applicable to food fermented by the addition of microorganisms, starter cultures, since more robust starter cultures can also be produced by growth on resistant starch medium and/or addition of resistant starch to subsequent products as described above.

Disclosure of the Invention

In a first aspect, the present invention consists in an improved microbial preparation having increased growth and/or yield potential and/or an increased survival rate/recovery in a food, feed, nutraceutical or pharmaceutical products.

Preferably, the microbial preparation comprises microbes grown in media based on, or containing, resistant starch. One form of resistant starch particularly suitable for the present invention is high amylose starch containing resistant starch and/or dietary fibre, particularly high amylose maize starch.

In use, the preparation may be mixed with additional resistant starch to further enhance the growth and/or yield potential of the microbes and/or the increased survival rate/recovery of the microbes in food, feed, nutraceutical or pharmaceutical products.

The microbes in the improved microbial preparation according to the first aspect of the present invention are particularly resistant to stresses including aeration, sheer, freeze drying, freezing, drying including high, medium and low water activity, elevated temperatures, low temperatures, pressure and pressure fluctuations, low pH, high pH, bile acids, moisture, or combinations thereof.

The microbial preparation according to the present invention is particularly suitable as a probiotic or a starter culture.

In a second aspect, the present invention consists in a process of preparing an improved microbial preparation having increased growth and/or yield potential and/or an increased survival rate/recovery in a food, feed, nutraceutical or pharmaceutical product, the process comprising culturing the microbes in media based on, or containing, resistant starch and harvesting the cultured microbes.

In a third aspect, the present invention consists in an improved microbial preparation having increased growth and/or yield potential and/or an increased survival rate/recovery in a food, feed, nutraceutical or pharmaceutical product prepared by the process according to the second aspect of the present invention.

In a fourth aspect, the present invention consists in an improved food, feed, nutraceutical or pharmaceutical product containing microbes having increased growth and/or yield potential and/or an increased survival rate/recovery, the food, feed, nutraceutical or pharmaceutical product including a microbial preparation according to the first or third aspects of the present invention.

In a preferred embodiment, the food product also includes resistant starch so as to further enhance the growth and/or yield potential and/or an increased survival rate/recovery of the microbes.

In a fifth aspect, the present invention consists in the use of resistant starch in microbial culture media to produce microbes having increased growth and/or yield potential and/or an increased survival rate/recovery in a food, feed, nutraceutical or pharmaceutical product.

One form of resistant starch particularly suitable for the present invention is high amylose starch containing resistant starch and/or dietary fibre, particularly high amylose maize starch.

The invention also includes microorganisms of different strains or species, including non-starch utilisers, to interact and demonstrate improved growth and/or activity in the large bowel.

In a preferred embodiment of the first, second, third and fourth aspects of the present invention, the microbial preparations are starter cultures or probiotic preparations which can be liquid, frozen or dried. The preparations may also include food and feed products containing other microbial additives. These products include fluid-based or solid-based products. Fluid-based food products include milk-based products where the edible ingredient is one or more milk-based ingredients including whole milk, milk solids, milk fat, cream, non-fat dried milk, any other component or derivative from milk that can be used in milk-based products, water-based fluids, cereal and plant extracts such as soy-based beverages and additives. Solid-based food products include snack bars, breakfast cereals, bread, confectionary, extruded food products, muesli bars, coated food products, tablets, food additives, health supplements, and pharmaceutical preparations.

The food products according to the fourth aspect of the present invention include any food product that is suitable to contain and deliver probiotic microorganisms. Examples include, but not limited to, food stuffs, fruit beverages, water ices, confectionary, coatings or covertures, yoghurts, yoghurt drinks, unfermented drinks, flavoured milk drinks, modified milk drinks, ice-creams, and dairy desserts.

Standard methods employed by the art can be used to prepare the food, feed, nutraceutical or pharmaceutical products according to the fourth aspect of the present invention. The resistant starch may be added separately, in combination with one or more of the ingredients that form part of the food product. The resistant starch when added separately may interact positively and/or synergistically with other ingredients in the food, feed, nutraceutical or pharmaceutical products.

The microbe is preferably a probiotic microorganism or starter culture, more preferably a lactic acid-producing or lactic acid bacteria including lactobacillus, a bifidobacterium strain, or mixtures thereof. Any microbe of

gastrointestinal origin or related microbe, however, may also be suitable for the present invention.

The increase in survival rate of the microbes in the food, feed, nutraceutical or pharmaceutical product relates to an increase over the expected survival rate of the same microbe in a similar food, feed, nutraceutical or pharmaceutical product that does not contain the resistant starch-grown microbes.

In a preferred form, the resistant starch is the Hi-maize™ and Culture Pro™ range of resistant starch products. The resistant starch can be used in growth media at a concentration of about 0.01 to 10% (w/w) and in subsequent additions during preparation of the microbial preparations and in liquid food, feed, nutraceutical or pharmaceutical products. Preferably the resistant starch is used at 0.1 to 5% w/w and more preferably at about 1% (w/w). The starch containing resistant starch and/or dietary fibre can be used in dry food, feed, nutraceutical or pharmaceutical products and microbial preparations at a concentration of about 0.1 to 90% (w/w) total product or preparation. Preferably, the starch is used at about 1 to 10% (w/w).

Resistant starch has been found to be particularly suitable in fluid-based foods at a concentration of 0.1 to 5% w/v, in solid-based foods at 0.1 to 15% w/w, and in feed, nutraceutical or pharmaceutical products at 0.1 to 95% w/w.

A further advantage of the use of resistant starch is that additional resistant starch can also be added at any stage during the processing of the food, feed, nutraceutical or pharmaceutical product. The properties of the resistant starch are not adversely effected by the processes involved in producing processed products. One distinct advantage is that there is no need to add the resistant starch in sterile form at the end of the process. The product can undergo pasteurisation or the like without the concern of adversely effecting the starches' properties.

As used in this specification, "resistant starch" includes those forms defined as RS1, RS2, RS3 and RS4 as defined in Brown, McNaught and Moloney (1995) Food Australia **47**: 272-275. Either modified or unmodified resistant starches or mixtures thereof can be used in the present invention.

Chemical modifications, such as oxidation, cross-bonding, etherification, esterification, acidification and the like are well known in this art as being suitable chemical treatments. Similarly, other modifications can

be induced physically, enzymically or by other means well known to those skilled in the art.

It may also be useful to modify the degree of enzyme susceptibility of the resistant starch by altering the conformation or structure of the starch. Examples include acid or enzyme thinning and cross bonding using di-functional reagents, heat/moisture treatment and thermal annealing. Modification of the starch may also be carried out by manipulation of the crystalline nature of the starch. Such modification methods are known to the art and starches produced by these methods would be suitable for the present invention.

Preferably the resistant starches are derived from corn (maize). It will be appreciated, however, that other sources of resistant starch could be used in the present invention. Examples include cereals like sorghum, wheat, barley, oats and rice, tubers like potatoes and tapioca, legumes such as peas, and others including starches derived from genetically modified plant species.

As used herein, Hi-maize™ and Culture Pro™ refers to products obtained from high amylose starch containing over 70% amylose obtained from Starch Australasia Limited.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples.

Disclosure of the Invention

The present inventors have found that the inclusion of resistant starch to microbial growth media and optionally in subsequent stages of production of microbial preparations and food/feed containing the resistant starch-grown microbes resulted in a surprising and unexpected increase in growth recovery and/or survival of the microbes during production and storage of the preparations and products.

EXAMPLE 1

Bifidobacterium strain Lafti™ 13B was anaerobically grown on a basal agar medium (BM) supplemented with 1% w/w of either glucose or resistant starch (Culture Pro™). After growth, cells were harvested from the plates using phosphate buffered saline (PBS) and aliquots were mixed with either PBS or PBS containing the starch granules (10% w/w). Aliquots of the mixtures were freeze dried. The susceptibility of the Lafti™ 13B cells to low pH was evaluated by adding the bacterial mixtures before freeze drying and rehydrated after freeze drying, to glycine buffer at a pH of 3.5. The viable cells were enumerated by determining colony forming units using Tryptone Yeast Extract Peptone plates (TYP) when added to pH 3.5 and after 3 hours. The reduction of viability over the 3 hours is presented in Table 1. It was noted that cells grown in the presence of starch were more resistant and that the inclusion of the starch further enhanced the resistance.

Table 1

	Presence of starch after growth	**Reduction of viability**
Glucose grown cells	-	26×10^2
Glucose grown cells	+	15×10^2
Starch grown cells	-	26
Starch grown cells	+	5

EXAMPLE 2

Bifidobacterium strain Lafti™ 13B was pre-cultured in Basal broth (BM) supplemented with 1% w/v glucose or high amylose maize starch granules (Culture Pro™). Anaerobically grown cultures were inoculated (10 µl) onto BM agar or into broth, both media containing 1% w/w glucose or Culture Pro™. Plates were either spot inoculated or spread and then incubated anaerobically for 48 hr. Growth in broth or cells harvested from spread plates were quantified by enumerating the colony forming units (CFU). Growth on spot-inoculated plates was quantified by measuring the size of the colony as well as the size of the cleared zone around the colony which was indicative of utilisation of the starch by the Bifidobacterium cells. It was noted that Lafti™ 13B grew more rapidly on starch-containing medium when

pre-cultured using starch-containing medium and produced larger colonies and cleared zones on agar plates containing starch. Furthermore, the yield was greater from starch-containing media, compared to glucose media, for cells pre-cultured in both control broth (glucose) and starch broth.

RESULTS

Recovery of viable microorganisms after growth in the presence of starch was higher and more rapid than glucose controls.

Growth in starch media enhanced the yield of microorganisms after exposure to stress conditions, for example, low pH, bile acids, acids, heat, moisture, pressure, freeze drying, spray drying, singly or in combination.

Pre-culture in starch medium prior to growth in starch medium enhanced recovery/survival after exposure to stress conditions as outlined above, as well as enhancing yield.

Growth in starch medium and then addition of starch enhanced resistance to stress conditions as outlined above.

USES

(i) The invention can be applied to all situations for which probiotic microbes may be used, including use as prophylactic and therapeutic agents as well as in food and feed compositions for the benefit of the host.

(ii) The probiotic microorganisms can be grown in the starch-based medium and used directly or combined with additional starch after growth. These probiotic suspensions may be used directly or after freezing and/or drying in the absence or presence of further additives .

(iii) The probiotic microorganisms described in (ii) above, can be added to foods and feeds either during or at the end of production.

(iv) In addition to the foods and feeds described in (iii) above, the starch may also be added to the food or feed prior to or after addition of the probiotic microorganisms.

(v) The invention also applies to microorganisms, including starter cultures, used for production of fermented foods wherein these microorganisms are grown in starch-based media and optionally mixed with additional starch after growth, freezing and/or drying, thereby enhancing survival of the microbes. When these microbes are added as an ingredient, additional starch may be added to the food before, during or after production.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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